

In the Specification

Please insert the following after the title and before the Field of the Invention:

Cross-Reference to Related Applications

The present application is a national application based on PCT application PCT/AU2003/001448, filed November 3, 2003, which claims priority to Australian application 2002952533, filed November 4, 2002, which are incorporated herein by reference to the extent permitted by law.

Please insert the following at the end of the section entitled "Methods of the invention" on page 21:

Aspect 1. A method for enhancing the solubilisation of at least one proteinaceous macromolecule in a biological sample without inducing substantial acid hydrolysis of said proteinaceous macromolecule, said method comprising incubating the biological sample in a solubilisation reagent at a pH between about pH 1.0 and about pH 6.0.

Aspect 2. The method according to aspect 1, wherein the solubilisation reagent has a pH of between about pH 1.0 and about pH 6.0.

Aspect 3. The method according to aspects 1 or 2, wherein the biological sample is incubated in a solubilisation reagent at a pH of between about pH 2 to about pH 5.

Aspect 4. The method according to aspects 1 or 2, wherein the biological sample is incubated in a solubilisation reagent at a pH of between about pH 3 to about pH 4.

Aspect 5. The method according to aspects 1 or 2, wherein the biological sample is incubated in a solubilisation reagent at a pH of between about pH 2 to about pH 3.

Aspect 6. The method according to aspects 1 or 2, wherein the proteinaceous macromolecule is solubilised in the presence of an aqueous acidic reagent selected from the group consisting of an organic acid solution, inorganic acid solution, acidic buffer, amino acid solution or a mixture thereof

Aspect 7. The method according to aspect 6, wherein the organic acid is selected from the group consisting of an ascorbic acid, carboxylic acid and polycarboxylic acid, or a derivative or mixture thereof.

Aspect 8. The method according to aspect 7, wherein the carboxylic acid is selected from the group consisting of formic acid, acetic acid, propionic acid, butyric acid, valeric acid, and benzoic acid, or a derivative or mixture thereof.

Aspect 9. The method according to aspect 7, wherein the polycarboxylic acid is selected from the group consisting of oxalic acid and citric acid or a derivative or mixture thereof.

Aspect 10. The method according to aspect 6, wherein the inorganic acid is selected from the group consisting of phosphoric acid, and orthophosphoric acid or a derivative or mixture thereof.

Aspect 11. The method of aspect 6, wherein the acidic buffer comprises acitrophospha buffer.

Aspect 12. The method of any one of the preceding aspects wherein the solubilisation reagent comprises a chaotropic agent.

Aspect 13. The method of any one of the preceding aspects wherein the solubilisation reagent comprises a detergent.

Aspect 14. The method according to any one of the preceding aspects wherein the biological sample is subjected to a physical or chemical means to disrupt the biological sample.

Aspect 15. The method according to any one of the preceding aspects, further comprising recovering the at least one solubilised proteinaceous macromolecule.

Aspect 16. The method according to aspect 15, wherein the solubilized proteinaceous macromolecule is recovered by performing a process comprising precipitating the at least one solubilised macromolecule.

Aspect 17. The method according to any one of the preceding aspects, wherein the solubilized proteinaceous macromolecule is recovered by performing a process comprising precipitating and resuspending the protein precipitate.

Aspect 18. The method according to any one of the preceding aspects further comprising reducing and alkylating the resuspended protein precipitate.

Aspect 19. A method of solubilising at least one proteinaceous macromolecule in a biological sample without inducing substantial acid hydrolysis of said proteinaceous macromolecule, said method comprising:

(i) subjecting the biological sample to a physical or chemical means to disrupt said biological sample and incubating the biological sample in the presence of a reagent at a pH between about pH 1.0 and about pH 6.0 to thereby solubilize at least one proteinaceous macromolecule in the biological sample; and

(ii) performing one or more processes selected from the group consisting of:

(a) recovering the solubilized proteinaceous macromolecule by performing a process comprising precipitating one or more proteins in the extract at (i) to thereby precipitate at least the solubilised proteinaceous macromolecule and resuspending the precipitated proteinaceous macromolecule;

(b) reducing and alkylating the solubilized proteinaceous macromolecule at (i) or the resuspended proteinaceous macromolecule at (ii)(a); and

(c) subjecting the solubilized proteinaceous macromolecule at (i) or the resuspended proteinaceous macromolecule at (ii)(a) or the reduced and alkylated proteinaceous macromolecule at (ii)(b) to a resolving means for a time and under conditions sufficient to resolve the proteinaceous macromolecule from other macromolecules present in the biological sample and then identifying the resolved proteinaceous macromolecule.

Aspect 20. A method of solubilising at least one proteinaceous macromolecule in a biological sample without inducing substantial acid hydrolysis of said proteinaceous macromolecule, said method comprising:

(i) subjecting the biological sample to a physical or chemical means to disrupt said biological sample, thereby producing a proteinaceous extract;

(ii) incubating the proteinaceous extract in the presence of a reagent having a pH between about pH 1.0 and about pH 6.0 to thereby solubilize at least one proteinaceous macromolecule in the extract; and

(iii) performing one or more processes selected from the group consisting of:

(a) recovering the solubilized proteinaceous macromolecule by performing a process comprising precipitating one or more proteins in the extract at (ii) to thereby precipitate at least the solubilised proteinaceous macromolecule and resuspending the precipitated proteinaceous macromolecule;

(b) reducing and alkylating the solubilized proteinaceous macromolecule at (ii) or the resuspended proteinaceous macromolecule at (iii)(a); and

(c) subjecting the solubilized proteinaceous macromolecule at (ii) or the resuspended proteinaceous macromolecule at (iii)(a) or the reduced and alkylated proteinaceous macromolecule at (iii)(b) to a resolving means.

Aspect 21. The method according to aspects 19 and 20, wherein the resolving means comprises a proteomic technique selected from the group consisting of: two-dimensional electrophoresis, one-dimensional electrophoresis, HPLC and liquid chromatography-mass spectrometry (LC-MS) or a combination thereof.

Aspect 22. The method according to any one of aspects 19-21, further comprising the following steps:

iv) digesting the resolved macromolecule, and

v) identifying the digested macromolecule by mass-spectrometry.

Aspect 23. The method according to aspect 22, wherein the macromolecule is digested by at least one proteolytic enzyme.

Aspect 24. A kit for enhancing solubilisation of a proteinaceous macromolecule a biological sample without inducing substantial acid hydrolysis of said proteinaceous macromolecule, the kit comprising a solubilisation reagent to solubilise at least one macromolecule in a biological sample, wherein the solubilisation reagent has a pH of about pH 1 to about pH 6 and optionally comprising directions to solubilise and/or recover a macromolecule in a biological sample, and/or directions to resolve a macromolecule in a biological sample.

Aspect 25. A proteinaceous macromolecule solubilised by the method according to any one of aspects 1 to 23.

Aspect 26. Use of an acidic reagent having a pH of about pH 1 to about pH 6 in the preparation of an solubilisation reagent solution for use in solubilising a proteinaceous macromolecule from a biological sample, without inducing substantial acid hydrolysis of said proteinaceous macromolecule.